**2475-Pos Board B245**
**Dynamic Switch Between Assembly States of the Human Bloom's Syndrome Helicase during Homologous Recombination**
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During normal cellular functioning, various endogenous and exogenous DNA-damaging effects arise, including the production of reactive oxygen species and UV irradiation. Double-stranded DNA breaks (DSB) represent one of the most toxic forms of DNA damage, which can be lethal for the cell or induce malignant transformation. Homologous recombination (HR) is an essential and evolutionarily conserved pathway for the error-free repair of DSBs. However, excessive HR can also lead to harmful large-scale genome rearrangements. HR events are therefore tightly regulated. A key player in HR is the human Bloom's syndrome helicase (BLM). During HR, BLM exerts various molecular activities. Based on ensemble biochemical and single-molecule AFM studies we show that the different actions of BLM during HR take place in different oligomeric forms of the enzyme. During single-stranded DNA translocation, which serves as a basis for quality control of HR via disruption of Rad51 nucleoprotein filaments, BLM functions as a monomer. Contrary, more complex DNA structures resembling later HR intermediates, including D-loops and Holliday junctions, induce dimerization and higher-order oligomerization of BLM. The results indicate that BLM exists in a dynamic equilibrium between different assembly states, which is modulated by the structure of DNA intermediates encountered during HR.

**2476-Pos Board B246**
**Single-Molecule Studies of Restriction Endonuclease Kinetics**
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Under optimal conditions, restriction endonucleases are capable of mediating remarkably specific DNA cleavage. This quality makes the restriction endonuclease an indispensible tool for genetic modification and manipulation. However, the mechanism by which restriction endonucleases effectively discriminate between their cognate site and other DNA sequences is not fully understood. Under certain conditions, many restriction endonucleases display "star activity" - relaxed specificity resulting in DNA cleavage at sequences that differ from their normal recognition sequence - but the mechanism by which specificity is relaxed is not fully understood. Although at least 600 of the almost 4000 restriction endonucleases that have been identified are commercially available in purified form, DNA cleavage kinetics of only a few of these enzymes have been studied in detail. We have developed a fluorescence-based approach with which we can track the progress of the cleavage reaction in real time, and simultaneously determine the values of the kinetic constants for a particular restriction endonuclease at a specific sequence. Modeling restriction endonuclease-mediated DNA cleavage as a Michaelis-Menten-like process, we expected reaction rates to display a hyperbolic dependence on substrate concentration, but our measurements deviate from this dependence, especially under conditions associated with increased star activity (such as low ionic strength). These observations suggest that substrate inhibition may be a part of the reaction mechanism under normal conditions, and that star activity may be a result of an increase in the population of this pathway. Using high density arrays of femtoliter-sized reaction vessels created by selectively etching bundled optical fibers, we can observe the cleavage activity of hundreds of individual restriction endonuclease molecules in solution. By characterizing the population distribution of single-enzyme turnover rates under a variety of conditions, we hope to gain insight into the reaction mechanisms of both specific cleavage and star activity.

**2477-Pos Board B247**
**G4 Quadruplex Recognition in the Human DEAH-Box Helicase RHAU**
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G4 quadruplexes of nucleic acids are very stable stacks of four guanylates that are associated in co-planar fashion via hydrogen bonds. Monovalent cations such as potassium or sodium are bound in the center of the quartet of guanyl groups. G4 quadruplexes can only be unwound enzymatically in vivo and this requires an energy source like ATP. G4 quadruplexes provide therefore the means to regulate nucleic acid specific cellular processes such as telomerase activity or gene expression. The recently discovered DEAH-box helicase RHAU, also known as DHX36, binds to G4 DNA and RNA quadruplexes and unwinds them. G4 quadruplex recognition occurs in a short stretch of amino acids in the N-terminal domain of RHAU, named "RHAU specific motif". Using a combination of biophysical methods such as Circular Dichroism, Dynamic Light Scattering, Size Exclusion Chromatography, Analytical Ultracentrifugation, Small Angle X-ray Scattering, Nuclear Magnetic Resonance and X-ray crystallography we are striving to shed light into how RHAU recognizes and binds G4 DNA and RNA complexes.

**2478-Pos Board B248**
**Single Molecule Observation of MicroRNA Processing Enzymes at Action**
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MicroRNAs (miRNAs) regulate mRNA expression via RNA interference. MicroRNA is generated when nuclear RNase III Drosha cleaves a long primary transcript liberating a hairpin precursor miRNA (pre-miRNA). The pre-miRNA is processed into a ~22 nt mature miRNA by cytoplasmic RNase III Dicer. In stem cells and certain cancer cells, the maturation process is interrupted when the pre-miRNA is hijacked by Lin28, a pluripotency factor, and the 3' end of the RNA is uridylated by a noncanonical polyA polymerase, TUT4.
With single molecule fluorescence microscopy, here we reveal the action mechanisms of human Dicer and TUT4 proteins at the molecular level. As it is difficult to prepare as large eukaryotic proteins as human Dicer and TUT4 proteins, we used a single molecule immunoprecipitation technique that we had recently developed [1].
We, for the first time, observed Dicer's cleavage activity in real time using single molecule FRET and revealed stepwise processes of the cleavage action. Next, we investigated how Lin28 interfered with Dicer's activity and mediated TUT4's uridylation of pre-miRNA. Our real-time analysis suggests that Lin28 functions as a processivity factor of TUT4 and TUT4 utilizes a looping mechanism when it attaches uridines to a recruited pre-miRNA.
Reference:
[1] K. H. Yeom, I. Heo, J. Lee, S. Hohng, V. N. Kim, C. Joo, (2011) "Single Molecule Approach to Immunoprecipitated Protein Complexes: Insights into MiRNA Uridylation" EMBO Reports, 12, 690-696.

**2479-Pos Board B249**
**Helicase-Initiated Assembly of the Primosome**
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A direct quantitative analysis of the initial steps in primosome assembly, involving PriA and PriB proteins and the minimal primosome assembly site (PAS) of phage ϕX174, has been performed using fluorescence intensity, fluorescence anisotropy titration, and fluorescence resonance energy transfer techniques. We show that two PriA molecules bind to the PAS at both strong and weak binding sites on the PAS structure without detectable cooperative interactions. Binding of the PriB dimer to the PriA - PAS complex dramatically increases PriA's affinity for the strong site, but only slightly affects its affinity for the weak site. Interactions are driven by apparent entropy changes, with binding to the strong site accompanied by a large unfavorable enthalpy change. The PriA - PriB complex, formed independently of the DNA, is able to directly recognize the PAS structure. Thus, the high-affinity state of PriA for PAS is generated through PriA - PriB interactions. The effect of PriB is specific for PriA-PAS association, but not for PriA-double-stranded DNA or PriA-single-stranded DNA interactions. Only complexes containing two PriA molecules can generate a profound change in the PAS structure in the presence of ATP. The obtained results provide a quantitative framework for initiation of primosome formation and elucidation of further steps in the assembly process.

**2480-Pos Board B250**
**Non-Equilibrium Topology Simplification by Type II Topoisomerases: A Test of Kinetic Proofreading**
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Type II topoisomerases are essential enzymes that modify DNA topology. These topoisomerases pass a double stranded segment (T-segment) of DNA though a transient double stranded break in a second segment (G-segment).